CLAIMS

1. A method of expressing a plurality of proteins encoded by a library of DNA vectors, wherein the library of vectors comprises a plurality of different vectors, each different vector comprising a different protein-encoding nucleic acid sequence, said nucleic acid sequence being operably linked to an expression-regulating region and optionally a secretion signal encoding sequence, the method comprising the steps of:

(a) providing a filamentous fungus having a phenotype characterized by growth in suspension and characterized by the production of transferable reproductive elements in suspension;

(b) stably transforming said filamentous fungus with said library of DNA vectors so as to introduce into each of a plurality of the individual fungi at least one heterologous protein-encoding nucleic acid sequence;

(c) culturing the transformed mutant filamentous fungi under conditions conducive to formation of transferable reproductive elements in suspension;

(d) separating from one another a plurality of transferable reproductive elements; and

(e) culturing into monoclonal cultures or monoclonal colonies the individual transferable reproductive elements, under conditions conducive to expression of the heterologous proteins encoded by the heterologous protein-encoding nucleic acid sequences.

2. A method of screening a plurality of proteins encoded by a library of DNA vectors for an activity or property of interest, comprising the steps of:

(a) expressing the plurality of proteins in monoclonal filamentous fungal cultures or monoclonal filamentous fungal colonies, by the method of claim 1; and

(b) screening individual clonal cultures or clonal colonies for the activity or property of interest.

3. A method of producing a DNA molecule encoding a protein having an activity or property of interest, comprising the steps of:

(a) expressing a plurality of proteins in monoclonal filamentous fungal cultures or monoclonal filamentous fungal colonies, by the method of claim 1;

(b) screening individual clonal cultures or clonal colonies for the activity or property of interest; and

(c) isolating DNA from a clonal culture or clonal colony exhibiting the activity or property of interest.

4. A method of producing the nucleotide sequence of a DNA molecule encoding a protein having an activity or property of interest, comprising the steps of:

(a) isolating DNA from a clonal culture or clonal colony exhibiting the activity or property of interest, by the method of claim 3; and

(b) sequencing said DNA.

5. A method of producing the amino acid sequence of a protein having an activity or property of interest, comprising the steps of:

(a) producing the DNA sequence of the protein having an activity or property of interest, by the method of claim 4; and

(b) converting said DNA sequence into an amino acid sequence.

6. A method of screening a plurality of monoclonal filamentous fungal cultures or monoclonal filamentous fungal colonies for a metabolite having an activity or property of interest, comprising the steps of:

(a) expressing a plurality of proteins in monoclonal filamentous fungal cultures or monoclonal filamentous fungal colonies, by the method of claim 1; and

(b) screening each individual clonal culture or clonal colony for the activity or property of interest.

7. A method of optimizing a protein's activity or property of interest, comprising the steps of:

(a) providing a library of vectors which comprise DNA sequences encoding mutant forms of the protein;

(b) providing a filamentous fungus having a phenotype characterized by growth in suspension and by the production of transferable reproductive elements in suspension;

(c) stably transforming said filamentous fungus with said library of DNA vectors so as to introduce into each of a plurality of individual fungi at least one heterologous protein-encoding nucleic acid sequence;

(d) culturing the transformed filamentous fungi under conditions conducive to the formation of transferable reproductive elements;

(e) separating from one another a plurality of transferable reproductive elements;

(f) culturing into clonal cultures or clonal colonies the individual transferable reproductive elements, under conditions conducive to expression of the heterologous proteins encoded by the heterologous protein-encoding nucleic acid sequences;

(g) screening each individual organism, clonal culture, or clonal colony for an expressed protein having the activity or property of interest;

(h) isolating one or more individual organisms, clonal cultures, or clonal colonies that express a protein exhibiting the activity or property of interest;

(i) mutating the DNA from the isolated individual organisms, clonal cultures, or clonal colonies that encodes the protein exhibiting the activity or property of interest;

(j) providing a library of vectors which comprise the mutated DNA sequences obtained in step (i); and

(k) repeating steps (b) through (g), until the property or activity of interest either reaches a desirable level or no longer improves.

8. The method of claim 7, further comprising between steps (h) and (i) the steps of: culturing one or more of the individual organisms, clonal cultures, or clonal colonies isolated in step (h); isolating the expressed protein exhibiting the activity or property of interest; and evaluating the isolated protein for the property of interest.

9. The method of claim 2, wherein the screening step is carried out by high-thoughput screening.

10. The method of claim 3, wherein the screening step is carried out by high-thoughput screening.

11. The method of claim 4, wherein the screening step is carried out by high-thoughput screening.

12. The method of claim 5, wherein the screening step is carried out by high-thoughput screening.

13. The method of claim 6, wherein the screening step is carried out by high-thoughput screening.

14. The method of claim 7, wherein the screening step is carried out by high-thoughput screening.

15. The method of claim 8, wherein the screening step is carried out by high-thoughput screening.

16. The method of any one of claims 1-15, wherein the fungus has a phenotype characterized by a culture viscosity, when cultured in suspension, of less than 200 cP at the end of fermentation when grown with adequate nutrients under optimal or near-optimal conditions.

17. The method of any one of claims 1-15, wherein the fungus has a phenotype characterized by a culture viscosity, when cultured in suspension, of less than 100 cP at the end of fermentation when grown with adequate nutrients under optimal or near-optimal conditions.

18. The method of any one of claims 1-15, wherein the fungus has a phenotype characterized by culture viscosity, when cultured in suspension, of less than 60 cP at the end of fermentation when grown with adequate nutrients under optimal or near-optimal conditions.

19. The method of any one of claims 1-15, wherein the fungus has a phenotype characterized by a culture viscosity, when cultured in suspension, of less than 10 cP at the end of fermentation when grown with adequate nutrients under optimal or near-optimal conditions.

20. The method of any one of claims 1-15, wherein the vectors comprise a fungal signal sequence.

21. The method of claim 20, wherein the fungal signal sequence is the signal sequence of a fungal gene encoding a protein selected from the group consisting of cellulase, β-galactosidase, xylanase, pectinase, esterase, protease, amylase, polygalacturonase and hydrophobin.

22. The method of any one of claims 1-15, wherein the vectors comprise a nucleotide sequence encoding a selectable marker.

23. The method of any one of claims 1-15, wherein the vectors comprise an expression-regulating region region operably linked to the protein-encoding nucleic acid sequence.

24. The method of claim 23, wherein the expression regulating region comprises is an inducible promoter.

25. The method of any one of claims 1-15, wherein the fungus is of the class Euascomycetes.

26. The method of claim 25 wherein the fungus is of the order Onygenales.

27. The method of claim 25 wherein the fungus is of the order Eurotiales.

28. The method of any one of claims 1-15, wherein the fungus is of the division Ascomycota, with the proviso that it is not of the order Saccharomycetales.

29. The method of any one of claims 1-15, wherein the fungus is of a genus selected from the group consisting of : *Aspergillus, Trichoderma, Chrysosporium, Neurospora, Rhizomucor, Hansenula, Humicola, Mucor, Tolypocladium, Fusarium, Penicillium, Talaromyces, Emericella* and *Hypocrea.*

30. The method of claim 29 wherein the fungus is of a genus selected from the group consisting of *Aspergillus, Fusarium, Chrysosporium, and Trichoderma.*

31.The method of claim 30, wherein the fungus is *Chrysosporium* strain UV18-25 having accession number VKM F-3631 D.

32. The method of claim 30, wherein the fungus is *Trichoderma longibrachiatum* strain X-252.

33. The method of claim 30, wherein the fungus is *Aspergillus sojae* strain pclA.

34. The method of claim 30, wherein the fungus is *Aspergillus niger* strain pclA.

35. The method of any of claims 1-15, wherein the expressed protein to biomass ratio is at least 1:1.

36. The method of claim 35, wherein the expressed protein to biomass ratio is at least 2:1.

37. The method of claim 36, wherein the expressed protein to biomass ratio is at least 6:1.

38. The method of claim 37, wherein the expressed protein to biomass ratio is at least 8:1.

39. The method of any of claims 1-15, wherein the transferable reproductive elements are individual fungal cells.

40. The method of any of claims 1-15, wherein the transferable reproductive elements are spores.

41. The method of any of claims 1-15, wherein the transferable reproductive elements are hyphal fragments.

42. The method of any of claims 1-15, wherein the transferable reproductive elements are micropellets.

43. The method of any of claims 1-15, wherein the transferable reproductive elements are

protoplasts.

44. A method for obtaining a protein having an activity or property of interest, comprising the steps of:

(a) screening a plurality of proteins encoded by a library of DNA vectors for an activity or property of interest, by the method of claim 2;

(b) culturing on appropriate scale the monoclonal culture or monoclonal colony expressing the activity or property of interest, under conditions conducive to expression of the heterologous proteins encoded by the heterologous protein-encoding nucleic acid sequences; and

(c) isolating the expressed protein.

45. A method for obtaining a protein having an activity or property of interest, comprising optimizing the activity or property of interest by the method of claim 7 or claim 8, culturing on an appropriate scale an individual organism, clonal culture, or clonal colony isolated in the final step (h), and isolating the expressed protein from the culture.

46. A method of making a library of transformed filamentous fungi, comprising the steps of:

(a) providing a filamentous fungus having a phenotype characterized by growth in suspension and characterized by the production of transferable reproductive elements in suspension; and

(b) stably transforming said filamentous fungus with a library of DNA vectors so as to introduce into each of a plurality of the individual fungi at least one heterologous protein-encoding nucleic acid sequence;

wherein the library of DNA vectors comprises a plurality of different vectors, each different vector comprising a different protein-encoding nucleic acid sequence, said nucleic acid sequence being operably linked to an expression-regulating region and optionally a secretion signal encoding sequence.

47. A library of transformed filamentous fungi, prepared by the method of claim 43.

48. A method for obtaining a transformed filamentous fungal host expressing a protein having an activity or property of interest, comprising the steps of:

(a) screening a plurality of proteins encoded by a library of DNA vectors for an activity or property of interest, by the method of claim 2; and

(b) isolating the monoclonal culture or monoclonal colony expressing the activity or property of interest.